REMARKS

Claims 1 and 3-48 are pending in the application, with claims 9, 11, 12, 15, and 21-46 withdrawn by the Examiner under 37 C.F.R. § 1.142(b). Claims 1, 3-8, 10, 13, 14, 16-20, 47 and 48 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. Also, claims 5 and 8 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner also stated that the application is not in compliance with the Sequence Rules.

Claims 1 and 16 are amended to improve the syntax of the claims. Claim 8 is amended to replace the term "compound" with the term "composition" for which there is antecedent basis. Claim 47 is amended to clarify the nature of IDO+ APCs, and to describe that the tumor or tumor-draining lymph node is one that expresses mip-3α; support for the amendment of claim 47 is provided by previously presented claims 16 and 19. Claim 48 is amended to correct the dependency of the claim. The specification is amended to provide SEQ ID NO: 1 as the identifier for the amino acid sequence on page 44, and to correct typographical errors. Accordingly, no new matter is added by the amendment of the claims or the specification.

Compliance with the Sequence Rules

The Examiner stated that the application is not in compliance with the Sequence Rules in that the amino acid sequence presented on page 44, line 10, is not identified by a sequence identifier. Applicants have amended the specification to denote that this sequence is SEQ ID NO: 1. Also, Applicants have submitted to Mail Stop Sequence a Sequence Listing as required under 37 C.F.R. 1.182 in both paper and electronic form. Applicants respectfully assert that the corrections to the specification, and the submission of a Sequence Listing place the specification in form that is in compliance with the sequence rules.

The Rejection of Claims Under 35 U.S.C. § 112, First Paragraph, Is Traversed Or Rendered Moot

A. Prima Facie Enablement

The Examiner rejected claims 1, 3-8, 10, 13, 14, 16-20, 47 and 48, as failing to comply with the enablement requirement. The Examiner stated that:

> The specification teaches that tolerance inducing APC express elevated levels of indoleamine 2, 3-dioxygenase (IDO) (page 8, lines 27-30). The specification teaches that tolerance-inducing cells may express a variety of proteins on their surface including chemokine receptor CCR6. MIP-3a is the known ligand for CCR6. The specification teaches that certain tumors express the chemotaxis factor MIP-3a (page 9, lines 23-27).
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> The specification teaches abnormal infiltration of IDO + cells in samples of tumor and tumor-draining lymph nodes from patients with malignant melanoma (pages 48-50). The specification teaches MIP-3a expression in malignant melanoma (pages 51, lines 3-12).

Office Action at pages 4-5.

The Examiner further stated, however, that "[t]he specification never disclosed if the tumor and tumor-draining lymph node samples from patients with malignant melanoma expressed receptor CCR6." Office Action at page 5. Applicants respectfully note that it is the IDO+ APCs that express CCR6, and not the tumor cells. Instead, the tumor cells express mip-3α. Thus, Applicants have discovered that CCR6 is preferentially expressed on IDO+ APCs (Figure 3), and that such IDO+ cells are tolerogenic in that they are markedly less efficient at stimulating T-cell proliferation than APCs that do not express elevated IDO (Figure 4). Also, Applicants' specification shows that IDO+ APCs are recruited to tumors *in vivo*, and that mip-3α is expressed *in vivo* by several different types of cancer cells, including the tumors have recruited IDO+ APCs (Figures 5 and 6). Additionally, even if the tumor cells were to express CCR6, it would not be problematic as application of an antibody would not be expected to enhance growth of a CCR6-expressing tumor, but to slow the growth and spread of the tumor.

The Examiner stated that "CCR6 receptor expression on IDO+ APC must be available in sufficient quantity and in appropriate context for a reasonable probability of immunorecognition by the CCR6 antibody (administered composition)." Office action at page 5. The ability of CCR6 antibodies to block the migration of IDO+ cells in a mip-3α

gradient is shown in the experiments of Example 9 (Figure 8). Also, it is known that mice with a targeted disruption of the CCR6 gene fail to recruit myeloid DCs into the lymphoid tissue of the gut (Varona et al., J. Clin. Invest., 107:R37-45, 2001), indicating that CCR6 as expressed by myeloid DCs is key to recruitment of these cells in response to cytokines and that blocking CCR6 can prevent recruitment of the DCs.

The Examiner also stated that:

> [A]n effective agent must **selectively inhibit** the recruitment of cells. The specification fails to teach how an administered CCR6 antibody would discern between an IDO+ APC expressing the CCR6 receptor and a non-tolerogenic APC or a different cell expressing the CCR6 receptor. It is unclear if non-tolerogenic APC or other cells in a tumor or tumor-draining lymph node area express CCR6 receptor. The specification fails to teach how one would determine reduced recruitment of IDO+ APC to tumor or tumor-draining lymph node *in vivo*. . . . [or] how the reduction of recruitment of IDO+ APC affects the metastasis of a tumor.
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> . . .

Office Action at page 5 (emphasis in original).

Applicants are only required to enable the claimed invention. Applicants' claimed invention is a method to reduce recruitment of antigen presenting cells (APCs) that inhibit T-cell proliferation to a particular site in a subject, comprising administering a composition to the subject to reduce recruitment of IDO+ antigen-presenting cells (APCs) or their precursors to the site, wherein the site is determined to comprise recruitment of IDO+ APCs, and wherein IDO+ APCs or their precursors are cells that express elevated levels of indoleamine 2,3-dioxygenase (IDO). Applicants have shown that CCR6 expression is correlated with IDO expression in human APCs (Example 3; Figure 3), and that the induction of tolerance is highly associated with, and presumably caused by, IDO (Example 4: Figure 4). Applicants have further shown that the majority of non-tolerogenic human APCs do not express CCR6 (Figures 3 and 4). Thus, Fig 3A and 3B show that all of CD123+ cells are also IDO+ cells. Fig 3C shows that all CCR6+ cells are also CD123+ and IDO+, but that IDO-negative cells are primarily CCR6-negative. Thus, the data shows that both CCR6 and CD123 are expressed by IDO+ DCs. Fig 4B shows that CD123+ DCs (also CCR6+ and IDO+) are the only population capable of suppressing lymphocyte proliferation in an allogenic MLR. Fig 4B also shows that the

population of cells depleted of CD123+ cells (i.e. cells that are $CD123^-$, $CCR6^-$ and IDO^-) are not capable of significantly suppressing T cell proliferation in an MLR.

The selectivity of Applicants' procedure is based on the uniform correlation of IDO expression and CCR6 expression in tolerogenic APCs, as shown in the specification for human cells *in vitro*, and as verified in subsequent experiments for murine tolerogenic IDO+ DCs isolated from mouse tumor-draining lymph nodes (see e.g., Munn et al, 2004, J. Clinical Investigation 114:280-290, at page 287 and Figure 7D; submitted herein as part of a Supplemental IDS). In contrast, those APCs that are not tolerogenic are heterogeneous for CCR6, with most being CCR6-negative (as shown in Figure 3C of the specification for human APCs; see also page 287 and Figure 7D of Munn, 2004, at page 287 for murine APCs). Therefore, all IDO+ DCs will be selected against using Applicants' procedure, but only a minority of IDO-negative DCs would be expected to interact with a CCR6 antagonist.

Absolute specificity is not necessary for the proposed technique to be clinically useful. As described in Example 14 at page 58, lines 1-5, and exemplified by the data of Figure 13 of Applicants' specification, the IDO+ DCs from tumor-draining lymph nodes are dominant in their suppression. Additionally, this dominant suppression appears to be titratable, in that below a certain ratio the suppression is lost, as shown in Example 13 and Figure 12 of the specification. Therefore, if the CCR6+ IDO+ DCs can be eliminated or significantly reduced in number, the suppression by IDO can be reduced.

Additionally, it would not cause problems for the technique if a portion of IDO-negative DCs were also blocked in their recruitment by CCR6 antagonists, since there are many other IDO-negative DCs that are CCR6-negative and that would not be affected. Nor is there any reason to expect that a reduction of other CCR6 expressing cells to the tumor-draining lymph node would lead to non-specific effects, since the other CCR6+ cells in circulation (a subset of B cells and a subset of memory T cells) are not the cells of interest (i.e., resting or native T cells) for reducing tolerance to tumors in the tumor-draining lymph node. Also, as discussed above, even if the tumor cells were to express CCR6, it would not be problematic as application of an antibody would not be expected to enhance tumor growth, but to slow the growth and spread of the tumor. In some cases, the antibody may be administered directly to the tumor and/or tumor-draining lymph

node as a means to promote the selective inhibition of recruitment of IDO+ APCs to the tumor or tumor-draining lymph node. Thus, Applicants are not specifically claiming that the administered CCR6 antibody would discern between and IDO+ APC expressing the CCR6 receptor and a different cell expressing the CCR6 receptor, but that the antibody may be used in a method to reduce the IDO+ APCs present at the tumor or tumor-draining lymph node.

Nor do Applicants specifically claim a reduction in tumor metastases. For the record, however, Applicants respectfully assert that by reducing recruitment of tolerance-inducing IDO+ APCs to a tumor and/or tumor-draining lymph node, the subject's immune response can be promoted, thereby reducing the viability of the tumor and any tumor cells that may reach the lymph nodes and/or blood stream.

The Examiner also stated:

> The in vitro experimental data presented is clearly not drawn to subjects with tumors or tumor-draining lymph nodes.
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> . . .
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> [T]he administered agent (i.e., CCR6 antibody) must accomplish several tasks to be effective. It must be delivered into the circulation that supplies the tumor and interact at the proper site of action and must do so at a sufficient concentration and for a sufficient period of time. In addition the target cell must not have an alternate means of survival despite action at the proper site for the drug. In vitro assays cannot duplicate the complex conditions of in vivo therapy.

Office Action at pages 6-7.

Applicants respectfully note that the references cited by the Examiner as describing the unpredictability of *in vitro* cell culture assays for development of anti-cancer therapeutics are based on literature reviews and findings from the 1980s and early 1990s. Applicants specifically note that the Detmer reference is critiquing the use of cultured cancer cells; however, cultured cancer cells were not used in any of Applicants' assays. Also, the references cited by the Examiner do not take into account many recent successful cancer treatments that have been developed. For example, Applicants submit herewith as part of a Supplemental IDS examples of the successful use of monoclonal antibodies for treatment of cancer. Thus, Lin et al., describe that since the late 1990s, monoclonal antibodies continued to be developed as important therapeutic compounds

for the treatment of cancer and that there are now more than 400 monoclonal antibodies in clinical trials (Lin et al., Clin. Cancer Res, 2005, 11:129-138 at page 130, column 2). Also, Zangemeister-Wittke describes that by the end of 2003, the market for 17 different antibody-based products generated several billion in combined annual sales (Zangemeister-Wittke, Pathobiology, 2005, 72:279-86, Abstract). Additionally, Applicants provide additional references documenting the efficacy of several monoclonal antibodies for treatment of cancer including: (1) Rituximab for treatment of follicular lymphoma, mantle-cell lymphoma and diffuse large B-cell lymphoma (Coiffier, C. R. Biol., 2006, 329:241-54); (2) Trastuzumab, a monoclonal antibody against HER2 used for treatment of breast cancer and showing improved survival for patients (Piccart-Gebhart, et al., N. Engl. J. Med., 2005, 353:1659-72; see also Siamon et al., N. Engl. J. Med., 2001, 344:783-92); (3) Cetuximab, a monoclonal antibody against epidermal growth factor receptor (EGFR) used for treatment of squamous cell carcinoma of the head and neck (Bonner et al., N. Engl. J. Med., 2006, 354:567-78) and colorectal cancer (a solid tumor) (Cunningham et al., N. Engl. J. Med., 2004, 351:337-45) and showing reduced mortality for treated patients; (4) Bevacizumab, an antibody to vascular endothelial growth factor (VEGF) used for treatment of metastatic renal cell carcinoma (Yang, et al., N. Engl., J. Med., 2003, 349:427-34) and showing reduced progression of the disease for treated patients; (5) a B-cell specific anti-CD20 monoclonal antibody for treatment of lymphomas (Kaminski et al., N. Engl. J. Med., 1993, 329:459-65) and showing reduced progression of the disease for treated patients; and (6) the use of three monoclonal antibodies (anti-DR5, anti-CD40 and anti-CD137) in combination with TRAIL to stimulate tumor-specific effector CD8+ cells for the treatment of fibrosarcomas (Uno et al., Nat. Med., 2006, 12:693-698). The success of monoclonal antibody therapy against tumors demonstrates that monoclonal antibodies are able to reach the tumor target cells at a sufficient concentration and for sufficient period of time to exert a therapeutic effect.

The Examiner also asserted that for an antibody to be effective against tumors, "the target cell must not have alternate means of survival at the proper site of the drug." Office Action at page 7. Applicants respectfully assert that this requirement is not problematic for the claimed invention, since the anti-CCR6 antagonists proposed by the

Applicants do not target tumor cells directly, but are only used block or reduce the migration of IDO+ APCs to the tumor site or draining lymph node. Also, there is no indication that there is a population of IDO+ APCs that would be substituted for the CCR6+ IDO+ APCs, as the experiments provided in the specification and Applicants' subsequent studies (described in more detail below) have verified that the population of IDO+ APCs in humans are consistently CCR6+.

Furthermore, Applicants respectfully assert that the *in vitro* data presented by the Applicants is clearly correlated to, and supports the use of, using the methods of the invention as a means to reduce the recruitment of IDO+ APCs to tumors or tumor-draining lymph nodes. The Federal Circuit has held that "as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement is satisfied." *In re Fisher*, 427 F2d 833, 839 (CCPA 1970). Further, MPEP Section 2164.02, teaches that an *in vitro* or *in vivo* animal model example in the specification constitutes a "working example" if that example "correlates" with a disclosed or claimed method invention. It is noted that "if the art is such that a particular model is recognized as correlating to a specific condition, then it should be accepted as correlating unless the examiner has evidence that the model does not correlate." MPEP 2164.03.

The *in vitro* models used in the studies in the specification, showing that CCR6 is expressed on IDO+ APCs, and that mip-3α can induce migration of CCR6-expressing cells are based on models that are well-accepted by those in the art. There are several examples in the literature (submitted herein as part of a Supplemental IDS) in which the inhibition of cytokines and/or chemokines *in vitro* with monoclonal antibodies has been correlated to clinically relevant applications *in vivo*. For example, monoclonal antibodies that target TNFα *in vivo* were shown to inhibit the production of secondary cytokines using *in vitro* models of Rheumatoid arthritis (RA). Randomized, placebo-controlled, multi-center clinical trials of human TNFα inhibitors have subsequently demonstrated that such agents display a consistent and remarkable efficacy in controlling signs and symptoms in approximately two thirds of patients for up to 2 years (Feldman and Maini, Ann. Rev. Immunol., 2001, 19:163-196).

Also, similar studies have been used to study the effects of an anti-IL8 antibody on migration of neutrophils. It was found that an anti-IL8 antibody that was able to inhibit IL-8 mediated chemotaxis and activation of the rabbit IL-8 receptor *in vitro*, was also able to block endotoxin-induced recruitment of neutrophils *in vivo* in animals that had received *E. coli* endotoxin (see Broaddus et al, J. of Immunol., 1994, 152: 2960-2967).

Applicants respectfully note that the method of the present invention does not necessarily require the use of a monoclonal antibody, but that chemokine binding proteins or small molecule inhibitors of the interaction between CCR6 and its ligand, mip-3α, may be used to inhibit the migration of IDO+ DCs to the tumor. For example, the soluble CC chemokine-binding protein of vaccinia virus 35K has been shown to dramatically reduce atherosclerosis and vein graft remodeling. This chemokine-binding protein provides localized broad-spectrum CC chemokine inhibition *in vitro* and *in vivo* (see e.g., Bursill et al, J. Immunol., 2006: 177:5567-73). It was found that m35K-expressing cells no longer undergo CC chemokine-induced chemotaxis *in vitro* and can locally deplete the CC chemokines, RANTES and MIP-1α, from the medium. These *in vitro* results are correlated to *in vivo* studies where it was found that intraperitoneal injection of mice with an adenovirus-encoding m35K lead to a significant decrease in leukocyte recruitment into the peritoneal cavity un a sterile peritonitis model (Bursill et al., 2006).

Another example of a non-antibody antagonist used to inhibit chemokine-induced polymorphonuclear leukocyte (PMN) chemotaxis is repertaxin. Repertaxin is a non-competitive allosteric blocker of interleukin-8 (CXCL8/IL-8) receptors (CXCR1/R2), which prevents receptor signaling and human polymorphonuclear leukocyte (PMN) chemotaxis *in vitro* and *in vivo* (See Castilli et al., Biochemical Pharmacology, 2005, 69:385-394 at page 386, col. 1). It was found that the effects of repertaxin on PMN migration *in vitro* were correlated to the ability of the compound to block CXCL8-induced T lymphocyte and other activities related to leukocyte recruitment.

For at least these reasons, Applicants respectfully assert that the studies provided in the specification enable the use of anti-CCR6 antibodies to reduce recruitment of IDO+ APCs to tumors and/or tumor-draining lymph nodes *in vivo*. Thus, the Applicants

respectfully assert that the Examiner has not made a *prima facie* case that the claimed invention is not enabled under 35 U.S.C. § 112, first paragraph, and respectfully request that the rejection be withdrawn.

B. Secondary Considerations

As noted above, Applicants do not concede that the Examiner has established a *prima facie* case of non-enablement. Still, to further prosecution, Applicants provide secondary evidence of why the claimed invention is enabled by the specification.

Declaration of Dr. David Munn

i. In vitro model for chemotaxis

Applicants submit herewith a Declaration Under 37 C.F.R. § 1.132 that the *in vitro* model for chemotaxis used in Examples 8 and 9 of the specification is a well-accepted model of the recruitment of CCR6 expressing cells by mip-3α as it occurs *in vivo*. Thus, it is accepted practice in the field of chemokine research to use this type of chemotaxis assay to predict the results of chemokine-induced migration that occur *in vivo*. Examples of the use of this type of assay is provided in the following references: (1) Dieu et al., 1998, J. Exp. Med., 188:373-386; (2) Yang et al., 1999, Science 286: 525-528; and (3) Zou et al., 2001, Nature Medicine 7:1339-1346 (submitted herein as part of a Supplemental IDS).

ii. Correlation of in vitro models to in vivo findings

Additionally, the *in vitro* model of IDO+ APCs has been found to accurately predict biologically relevant properties of the IDO+ cells found *in vivo* in tumor-draining lymph nodes. Thus, the experiments in the specification show that: IDO+ APCs are poor stimulators of T-cell proliferation (Example 4); IDO+ APCs express CCR6 (Example 3); IDO+ APCs are found in mip-3α expressing tumors (Example 5) and tumor-draining lymph nodes (Examples 11 and 12); and IDO+ APCs suppression of T-cell proliferation is titratable (Example 13). The investigators have also shown similar results in subsequent experiments for mouse IDO+ DCs. Thus, it was shown that immunosuppressive, tolerance-inducing DCs isolated from the tumor-draining lymph nodes of mice preferentially express CCR6 and CD123. In contrast, non-suppressive IDO-negative DCs from the same nodes express much lower levels of CCR6 and CD123 (see e.g., page 287 and Figure 7D, Munn et al., 2004, J. Clin. Invest., 114: 280-290).

Also, it was found that murine IDO+ DCs isolated from tumor-draining lymph nodes suppress T cell responses *in vitro* in a manner similar to the inhibition shown for human CCR6+ DCs, and that such suppression was dominant over DCs that do not express IDO (e.g., Munn et al., 2004 at page 281 and Figure 2). Thus, the studies as described in the patent specification (i.e., the *in vitro* and *in vivo* data showing mip-3α induced chemotaxis of CCR6+ APCs; the ability of CCR6+ IDO+ APCs to reduce T cell proliferation; and the detection of IDO+ APCs and mip-3α expression in tumor samples isolated from patients) predicted the *in vivo* expression of CCR6 and CD123 by murine IDO+ DCs in tumor-draining lymph nodes and the tolerogenic nature of these cells.

Also, when the murine IDO+CCR6+ DCs were injected into new, tumor-free mice, it was found that these cells were able to create an antigen-specific anergy in the host T cells (see e.g., Munn, 2004 at pages 282-283; Munn, 2005, Immunity 22:633-642 at page 638). Development of such antigen-specific anergy was prevented by the administration of the IDO inhibitor, 1-MT. Thus, the human *in vitro* system described in the patent application correctly predicted the *in vivo* immunosuppressive and tolerogenic attributes of these cells.

Because these subsequent *in vivo* studies were performed using methods that were standard in the art and were enabled by the disclosure of the application as filed, Applicants respectfully asserts that the specification enables a method to reduce recruitment of antigen presenting cells (APCs) that inhibit T-cell proliferation to a particular site in a subject, comprising administering a composition to the subject to reduce recruitment of IDO+ antigen-presenting cells (APCs) or their precursors to the site, wherein the site is determined to comprise recruitment of IDO+ APCs, and wherein IDO+ APCs or their precursors are cells that express elevated levels of indoleamine 2,3-dioxygenase (IDO).

For at least these reasons, Applicants respectfully assert that the claimed invention is enabled under 35 U.S.C. § 112, first paragraph, and request that the rejection be withdrawn.

The Rejection of Claims Under 35 U.S.C. § 112, Second Paragraph, Is Traversed Or Rendered Moot

The Examiner rejected claims 5 and 8 under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner rejected claim 5 for using of the recitation "wherein the biological signal . . . comprises MIP-3a." The Examiner stated that "[a] signal is a current, image, voltage, etc. A signal may be conveyed, transduced, or transmitted through (or by) MIP-3a. It is unclear how a signal comprises MIP-3a." Office Action at page 9.

Applicants respectfully assert that the recitation that "the biological signal . . . comprises mip-3α" is not indefinite. The term biological signal is well-accepted in the art as being a term used to refer to any aspect of a biological system that is used to infer that the system is in a particular state. Although a biological signal may, in some cases, be a current, image or voltage, a biological signal may also comprise the expression of a particular protein, the modification of a protein or other biomolecule (e.g., DNA methylation, protein phosphorylation), the expression of a cytokine (e.g., mip-3α) or another compound. Thus, a biological signal may comprise the formation of a concentration gradient of a protein or proteins. Applicants attach herewith three publications or the abstracts thereof as describing the following as biological signals: (1) the pattern of DNA methylation (Ramchandani et al., 1999, "DNA methylation is a reversible biological signal," Proc. Natl. Acad. Sci. USA, 96:6107-6112); (2) extracellular adenosine levels (Basheer et al., 2000, "Adenosine as a biological signal mediating sleepiness following prolonged wakefulness," Biol. Signals Recept., 9:319-327); and (3) melatonin (Pand-Perumal et al., 2006, "Melatonin: Nature's most versatile biological signal?" FEBS J. 273:2813-2838) (submitted herewith as a Supplemental IDS).

The Examiner stated that claim 8 was indefinite as there was no antecedent basis for the limitation "wherein the compound." Office Action at page 9. Applicants have amended the claim to replace "compound" with "composition" for which there is antecedent basis.

Thus, Applicants respectfully assert that for the above-reasons, claim 5 and amended claim 8 are not indefinite under 35 U.S.C. § 112, second paragraph, and request that the rejection be withdrawn.

CONCLUSION

In view of the foregoing amendment and remarks, each of the claims remaining in the application is in condition for immediate allowance. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the outstanding rejections. The Examiner is respectfully invited to telephone the undersigned at (336) 747-7541 to discuss any questions relating to the application.

Respectfully submitted,

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